BioSystems 114 (2013) 8–24

Contents lists available at ScienceDirect

BioSystems

journal homepage: www.elsevier.com/locate/biosystems

On eukaryotic intelligence: Signaling system's guidance in the evolution of multicellular organization

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ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form 28 June 2013
Accepted 30 June 2013

Keywords:
Eukaryotic signaling system
Signaling pathways
Domain recombination
Cellular problem-solving
Cellular intelligence
Neuronal signaling
Postsynaptic proteome
Cell theory

ABSTRACT

Communication with the environment is an essential characteristic of the living cell, even more when considering the origins and evolution of multicellularity. A number of changes and tinkering inventions were necessary in the evolutionary transition between prokaryotic and eukaryotic cells, which finally made possible the appearance of genuine multicellular organisms. In the study of this process, however, the transformations experimented by signaling systems themselves have been rarely object of analysis, obscured by other more conspicuous biological traits: incorporation of mitochondria, segregated nucleus, introns/exons, flagellum, membrane systems, etc. Herein a discussion of the main avenues of change from prokaryotic to eukaryotic signaling systems and a review of the signaling resources and strategies underlying multicellularity will be attempted. In the expansion of prokaryotic signaling systems, four main systemic resources were incorporated: molecular tools for detection of solutes, molecular tools for detection of solvent (Donnan effect), the apparatuses of cell-cycle control, and the combined system endocytosis/cytoskeleton. The multiple kinds of enlarged, mixed pathways that emerged made possible the eukaryotic revolution in morphological and physiological complexity. The massive incorporation of processing resources of electro-molecular nature, derived from the osmotic tools counteracting the Donnan effect, made also possible the organization of a computational tissue with huge information processing capabilities: the nervous system. In the central nervous systems of vertebrates, and particularly in humans, neurons have achieved both the highest level of molecular-signaling complexity and the highest degree of information-processing adaptability. Theoretically, it can be argued that there has been an accelerated pace of evolutionary change in eukaryotic signaling systems, beyond the other general novelties introduced by eukaryotic cells in their handling of DNA processes. Under signaling system's guidance, the whole processes of transcription, alternative splicing, mobile elements, and other elements of domain recombination have become closely intertwined and have propelled the differentiation capabilities of multicellular tissues and morphologies. An amazing variety of signaling and self-construction strategies have emerged out from the basic eukaryotic design of multicellular complexity, in millions and millions of new species evolved. This design can also be seen abstractly as a new kind of quasi-universal problem-solving 'engine' implemented at the biomolecular scale—providing the fundamentals of eukaryotic 'intelligence'. Analyzing in depth the problem-solving intelligence of eukaryotic cells would help to establish an integrative panorama of their information processing organization, and of their capability to handle the morphological and physiological complexity associated. Whether an informational updating of the venerable "cell theory" is feasible or not, becomes, at the time being – right in the middle of the massive data deluge/revolution from omic disciplines – a matter to careful consider.

1. Introduction: The distinctive problem solving capabilities of eukaryotic cells

The main goal of this paper is to continue a previous effort focused on the signaling "intelligence" of the prokaryotic cell (Marijuán et al., 2010), which is now addressed towards the eukaryotic camp. Actually, both papers may be taken as a single attempt to review the whole molecular apparatuses in charge of organizing the systematic relationships that any living cell has to maintain with its inner/outer environment. Amidst the bewildering variety of

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http://dx.doi.org/10.1016/j.biosystems.2013.06.005

molecular components and signaling pathways of eukaryotes, and their dense connection with the rest of cellular subsystems, we will see that a new discussion on the hallmarks of cellular intelligence, biocomputationally updating the venerable "cell theory" (framed by T. Schwann, M. Schleiden, and R. Virchow almost two Centuries ago), and its amendment by the "Central Dogma" in the 60s, could be framed. Perhaps, in the same way than a new field of "artificial intelligence" was launched decades ago stemming out from the processing capabilities of computers, the bio-informational capabilities of cells would nowadays demand their own multidisciplinary arena.

At stake is whether the reflections of theoretical biology are keeping pace with the phenomenal accumulation of empirical/computational data taking place. Cellular signaling systems have been the subject of countless works in last two decades, either molecularly, computationally, systemically or synthetically, but always focusing on some particular pathways or networks, and very few works have attempted the description of an integrative panorama or drafted the large-scale insights needed to comprehend their evolutionary trajectory. Whether it is a plausible task or not, given the outmost complexity and heterogeneity of eukaryotic signaling, is another matter. Nevertheless even an imperfect synthesis may be a useful resource in a field which has to be trodden by researchers and scholars from a number of disciplines. Needless to say, the authors are well aware of the famous Schrödinger's warning:

> I see no other escape from this dilemma (lest our true aim be lost forever) than that some of us should venture to embark on a synthesis of facts and theories, albeit with second hand and incomplete knowledge of some of them—and at the risk of making fools of ourselves. (cited in Stonier, 1990).

What is distinctive in eukaryotic signaling systems? Being itself a 'composite' of other cellular systems, the eukaryotic cell was forced to handle its inner organization of processes in new ways that later on allowed a far more effective problem solving, to be based on specialization of cell types and communication through multiple pathways–networks. We might argue that prokaryotes had already used some of those very capabilities, or at least their incipient evolutionary traits, mainly towards the direct solution of *molecular assimilation* problems (in their encounter with environmental substances); while eukaryotes were to achieve a fascinating developmental complexity by evolving towards a quasi-universal solution of *molecular organization* problems. Explaining away this difference involves a new interpretation of cellular organization, but not only in evolutionary-biological terms, "computer terms" also become necessary, or better, a new "informational" explanatory ground should be established. As we will argue, the tight coupling among transcription, alternative splicing, domain recombination, and cell differentiation, all of them under signaling system's guidance, integrates an abstract problem-solving 'engine' that transcends the biomolecular realm. Rather than following analogies with Turing machines Danchin (2009) or with operating systems (Yan et al., 2010), we will discuss the eukaryotic self-construction and communication capabilities in a new way, starting from von-Neumann's views of self-constructing machines (Vedral, 2010). This discussion will be addressed later on, at the end of the paper, once the evolution, structure, aggregated functioning, and classes of pathways of eukaryotic signaling systems have been drafted.

Evolutionarily, the complexity of eukaryotic signaling – which excels in the electromolecular operations of nervous systems – did not arise from scratch. A good portion of the new signaling system was directly inherited from prokaryotes, but many other parts were invented through *bricolage* and were cobbled together with highly complex controlling apparatuses unrelated to prokaryotes. Functionally speaking, however, the relative simplicity attributed to prokaryotic cells is only apparent, at least concerning their signaling capabilities. As was discussed in a previous publication (Marijuán et al., 2010), these cells have only three main classes of component-system arrangements for signaling purposes; but they are instantiated in about *one or two hundred* different pathways for each cell, acting as independent or colligated channels for the entrance of external information. In contrast, the cells of complex eukaryotes, such as vertebrates and mammals, are endowed with several dozen classes of component-system arrangements (main signaling pathways), but they comprise *thousands* of specific molecular implementations in different tissues, particularly within the nervous system. Amidst all that complexity, however, there is a deep evolutionary sense and coherence in the way signaling pathways have been assembled in eukaryotes and particularly in the functions they perform for the development and physiology of the multicellular organism. Appropriately interrelating such diversity and coherence will be the goal of this paper.

Essentially, the cellular signaling system is in charge of receiving and interpreting the signals that the whole organism instantiates, and of modifying accordingly the developmental/physiological trajectory followed by the concerned cell. As we will see, an overabundance of transmembrane molecular mechanisms are continuously sensing the external milieu, subsequent amplification cascades are conducing and networking the external changes registered, and finally quite many different actuators are mobilized—often transcription factors and associated proteins in the nucleus, but also many other molecules in the cytoplasm. Along the differentiation process, the cell changes its state and advances its life cycle by continuously following the incoming signals from the whole organism, mixing them with its own inner controlling mechanisms. In a curious parallel with the nervous system, it is the signaling system itself which instructs the cell about what are the specific external/internal signals to follow—in theoretical biology both are considered as "anticipatory systems" (Rosen, 1985). When the cell changes its state or differentiates, its own signaling system changes subsequently, but it has already been changed beforehand and has prepared the cell to distinguish the appropriate signals from the non-pertinent ones. This guidance mission of signaling systems both on cellular self-construction and on evolutionary grounds is one of the central ideas of this paper.

In the sections which follow, we will discuss first about evolutionary origins, on why a plethora of signaling resources was evolved and how a recombination strategy was mainly followed in the assemblage of this crucial system (Section 2). Subsequently we will examine in detail the "four roots" of eukaryotic signaling: detection of solutes, detection of solvent, cell-cycle control, and the combined system of endocytosis and cytoskeleton (Section 3). Further, a chart of the main eukaryotic signaling paths will be produced (Section 4), including a prototypical pathway scheme, a functional grouping of the pathways with a simplified classificatory attempt of their roles in development and physiology (21 pathways and pathway's classes described in Table 1). A brief examination of neuronal signaling as a prototype of signaling complexity will take place in Section 5, where a number of signaling pathways will be described, concretely at the postsynaptic site of glutamatergic excitatory neurons. Finally, in Section 6, the discussion about signaling and the problem-solving organization of the eukaryotic cell will be retaken; some hints will be introduced about a new informational theory on cellular self-constructing intelligence.

2. How signaling resources were evolved in the transition from prokaryotic to eukaryotic

An information revolution took place in cellular systems around 1200 Mys ago. It was preceded and made possible by an energy revolution derived from the symbiotic capture of mitochondria, as

Table 1
The major eukaryotic signaling pathways (21 of them), highlighting the main mechanisms and functionalities.

Signaling system pathways	Components (to DNA-binding)	Main functions	References
	Early development and morphogenesis		
Wnt pathway (glycoproteins)	Frizzled and LDL co-receptors → β-catenin → TCF/LEF *Non-canonical*: *Wnt/Ca*$^{2+}$, *Wnt/PCP*	Cell proliferation, migration, polarity, neural differentiation, axonal growth. Ciliogenesis, craniofacial development and regeneration	Reya and Clevers (2005), Eisenmann (2005), Dale et al. (2009)
Hedgehog pathway (Hh proteins)	Ptc/Ptch1 → 7TM co-receptor Smo → Ci/Gli	Cell growth, tissue homeostasis, neural tube differentiation, loss of neural stem cells, embryonic formation, tumor initiation and growth	Bermann et al. (2002), Lum and Beachy (2004), Hausmann et al. (2009)
Notch pathway (Notch/Delta proteins)	DSL TM proteins → NICD → CBF1/CSL/Rbp-j → MAML1	Cell-fate specification, differentiation, self renewal, proliferation and apoptosis, stem cells maintenance, vertebrate segmentation	Aulehla and Herrmann (2004), Schwanbeck et al. (2011), Ersvaer et al. (2011)
TGFβ pathway (TGFβ superfamily proteins)	Ser/Treo kinase (type I y II) → SMAD cascade *Non-canonical*: *Ras*/*MAPKs* (*ERKs & SAPKs*)	Cell division, differentiation, migration and adhesion; neuronal development and remodeling, synapse formation and growth, programmed cell death, suppressor of carcinogenesis	Mulder (2000), Derynck and Zhang (2003), Roberts and Mishra (2005), Massagué and Gomis (2006)
Trk kinase pathway (neurotrophins)	p75^{NTR} → Small G proteins → Ras/MAPK *Non-canonical*: *PLC*, *PI3K*	Cell survival, proliferation, axon and dendrite growth and patterning, cytoskeleton assembly & remodeling, synaptic strength and plasticity	Huang and Reichardt (2003), Segal (2003), Reichardt (2006)
	Mid-development and organogenesis		
Integrin pathway (glycoprotein)	ECM proteins → FAK → Ras/Raf → MEK → ERK *Non-canonical*: *actin, JNK, AKT/PKB, RLC*	Differentiation, proliferation, cell shape and migration, cytoskeletal organization, maintenance and cell survival	Martin et al. (2002), Stevens and George (2005), Yee et al. (2008), Moser et al. (2009)
Cadherin pathway (glycoprotein)	PDZ-domain proteins → protein phosphatase → protein kinase → actin *Non-canonical*: Rho family GTPases, Wnt, RTK	Embryonic development, tissue morphogenesis and homeostasis. Synaptogenesis, synaptic plasticity and synapse morfogenesis	Wheelock and Johnson (2003), Arikkath and Reichardt (2008), Hulpiau and van Roy (2009), Yagi and Takeichi (2000)
Nuclear hormone receptor pathway	NHR (monomer/homodimer/RXRheterodimer) → HREs	Cell growth and cycle progression, apoptosis, hypothalamic–pituitary–adrenal axis, neuro-endocrine stress response, autonomic nervous system, development, metabolic homeostasis	Aranda and Pascual (2001), Beildeck et al. (2010)
Reelin pathway (glycoprotein)	ApoER2/VLDLR → DAB1 → SFK → NMDAR Non-canonical: Cdk5, BLBP/Notch1	Neuronal migration and positioning in the developing brain, modulation of synaptic plasticity, induction and maintenance of LTP, stimulation of dendrites and dendrites spine, migration of neuroblast and neurogenesis	Akopians et al. (2008), Niu et al. (2008), Durakoglugila et al. (2009), Chameau et al. (2009)
	Tissue physiology		
Guanylatecyclase pathway (hormones, toxins, free radicals, calcium)	GCs + NO → GMPc → PK/PDE cascades GCp → GMPc → PK/PDE cascades	Neuronal signal transduction, vascular smooth muscle relaxation, inhibition of platelet aggregation, electrolytic homeostasis	Lucas et al. (2000), Hofmann et al. (2006)
G-protein coupled receptor (large G proteins) pathways	Adenyl cyclase → cAMP → EPACs/PKA/CNG channels GPCRs + Src-related Kinase + PI3K + Shc → Ras → MAPK	Cellular responses to hormones and neurotransmitters, immune responses, cardiac and smooth muscle contraction and blood pressure regulation, proliferation, tissue remodeling and repair, inflammation, angiogenesis, normal cell growth and cancer	Gavi et al. (2006), Dorsam and Gutkind (2007), Rosenbaum et al. (2009)
	Electrical transmission		
Gap junctions	Hemichannels or connexones permanently opened allowing unspecific ionic flow	Direct electrical transmission between neurons (and also glial cells)	Kandel et al. (2000), Kelsell et al. (2001), Willecke et al. (2002)
Stretch-activated channels	Mechanotransducer channels sensing membrane stress and allowing non-specific ionic flow	Vibration sensing, pressure, stretch, touch, heat sensation, hearing, osmotic and blood pressure, and propioceptive sensation	Kung (2005), Purves et al. (2008), Yin and Kuebler (2010)

Table 1 (*Continued*)

Signaling system pathways	Components (to DNA-binding)	Main functions	References
Voltage-activated channels	Voltage sensor channels allowing specific ionic flow (sodium, potassium, calcium, chloride, proton)	Generation and transmission of electrical signals in central and peripheral neurons, glia, skeletal muscle, heart, kidney, vascular tone, hormonal control, etc.	Catterall (2000), Kandel et al. (2000), Alberts et al. (2002)
Ligand-gated channels	Transmembrane ion channels opened or closed in response to the binding of a ligand or neurotransmitter, and allowing selective ionic flow (sodium, potassium, calcium, chloride, proton)	The main neurotransmitters (glutamate, acetylcholine, GABA, glycine, serotonin, etc.) represent the basic controls of brain function and the most general means of communication between neurons	Kandel et al. (2000), Barry and Lynch (2005), Swijsen et al. (2009)
	Stress and criticality		
Toll-like receptors pathway	TLR(1-2, 4-13) *MyD88dependent* + IRAK kinases → TRAF6 → TAK1 → IKKs →NFkB	Apoptosis, cell mediated immunity, bacterial death, autoimmune diseases (inflammatory process).	O'Neill et al. (2003), O'Neill (2008), Kumar et al. (2009)
	TLR (3–4) *TRIF dependent* + TBK/RIP1 → IRF3/TAK1-NFkB		
Cytokine receptors (cytoplasmic tyrosine kinases) pathway	Type I: JAK → STATs → IRF → ISRE Type II: JAK → STAT1 → GAS	Inflammatory and immunological response, cell proliferation and hematopoiesis; regulated survival of injured neurons, neurite elongation, and re-establishment of neuronal connections	Miyajima et al. (1992), Valentino and Pierre (2006), O'Sullivan et al. (2007)
	Non-canonical: JAK → MAPK/PI3K/AKT		
Autophagy pathway	mTOR → ULK complex (ULK1, ULK2, mAtg13) → FIP200 → Class III PI3K complex (hVps34, Beclin 1, p150, Atg14L)	Stress survival and longevity, elimination and recycling of damaged cellular components generated in response to induced oxidative stress or during normal aging, promoting constant cellular renewal, cell growth, development, and homeostasis	Levine and Kroemer (2008), Mizushima et al. (2008), Yang and Klionsky (2010)
Apoptosis pathway	Bcl-2 family (Bax, bak, Noxa, PUMA) → cit. C, Smac/Diablo, Omi/HtrA2 → Caspase 9 & Efector Caspases	Embryogenesis and the maintenance of tissue homeostasis during the stage adult, pathological conditions or in healthy tissue (for example, its participation in the development of nervous system and immune, or neurodegenerative dementias)	Kolesnick and Golde (1994), Gamen et al. (1998), Brunelle and Letai (2009), Almeida et al. (2012)
	Non-canonical: TNF receptor Superfamily (CD95/Fas/Apo, TNF-R1), Sphingomyelin-Ceramide		
The hippo pathway	DCHS 1/2 → FAT4 → FRMD6/Mer/KIBRA → Mst1/2 → Mob1 → Lats1/2 → YAP/TAZ	Stem cell and progenitor self-renewal, cell proliferation, antiapoptosis Organ size control	Huang et al. (2005), Zhao et al. (2010), Pan (2010)
Complement cascade	Classical: IgG/IgM → C1q → C4/C2 → C3 → C5	Immunoregulatory functions (enhancing humoral immunity, modifying T cell immunity, shaping the development of the natural Ab repertoire, regulating tolerance to nuclear self Ags such as DNA and chromatin)	Thurman and Holers (2006), Stevens et al. (2007), Alexander et al. (2008)
	Non-canonical: Lectin: MBL → MASPs → C4/C2 → C3	Pathogenic role (initiation and regulation of inflammatory response, opsonization and phagocytosis, systemic organ ischemia/reperfusion) and autoimmune diseases	
	Alternative: C3-H_2O → Factor B/Factor D/Properdin → C5		

Margulis (1970) so forcefully argued in her theory of Endosymbiosis. The data are staggering: an average protozoan has nearly 5000 times more metabolic power than a single bacterium, and can support a genome several thousand times larger with more than two orders of magnitude in the energy devoted to expression and translation of each gene (Lane and Martin, 2010). Whereas prokaryotes had already made a start towards cellular complexity eukaryotic style, they could not exhibit more than one complex trait at a time, given the energy costs implied. Novel protein folds, far more protein interactions, and enlarged regulatory cascades were required for putting together the isolated complexity traits that bacteria had already explored but in too restricted a way: separate nucleus, dynamic cytoskeleton, endocytosis, linear chromosomes, introns and exons, massive intracellular and intercellular signaling, etc. The increase in protein repertoire by the eukaryote common ancestor was dramatic: It represented some 3000 novel gene families—the most intense phase of gene invention since the origin of life (Lane and Martin, 2010, p. 933):

> If evolution works like a tinkerer, evolution with mitochondria works like a corps of engineers.

A heavy investment in signaling resources was necessary in order to produce a new kind of life cycle amenable to controlled dissociation or *modularization* amidst the far more complex internal

and external happenstances. Most cellular functions had to change from a temporal context to a spatial one, tightly controlled by specific signals; while some functions were delayed or directly suppressed, others became augmented and specialized (Nedelcu and Michod, 2004). The decoupling of cell division from cell reproduction, organizing successive levels of "potency" along the developmental process, was one of the central achievements. The cell cycle became contingent on signals received from other cells, whereas in single cells these processes had no such dependence (Gerhart, 1999). Thus, the capability to keep cells in a quiescent state, facultatively and reversibly by way of signaling instances, is what made possible the advent of true multicellularity (Davidson, 2006, 2010).

Four main functional resources were used in the expansion of eukaryotic signaling systems—four "roots" that supported the fast branching of all the new complexity. Two of them were, so to speak, directly taken from the existing prokaryotic stock:

- Prokaryotic signaling pathways, actually devoted to *detection of solutes* (and comprising: receptors, protein kinases, phosphatases, and regulated transcription factors).
- Prokaryotic osmotic apparatuses counteracting the Donnan effect, actually devoted to *solvent sensing* (and comprising: stretch ion-channels, voltage ion-channels, ligand-gated channels, water transporters, and pumps).

Another two signaling avenues were related to new cellular subsystems supporting the enlarged eukaryotic complexity. Those controlling apparatuses in charge of modularizing the new deployment of cellular functions, progressively *co-opted* for signaling purposes, became irremediably (or better, "facultatively") linked to the emergent complex network of signaling pathways:

- The cell-cycle controlling system (hierarchies of protein kinases, checkpoints, cyclins, and protein degradation systems).
- The cytoskeleton plus the endocytic matrix (mechanical support, adhesion, and force-field detection on the one side; compartments, inner transportation, and vesicle formation in the other).

Strategic areas of prokaryotic metabolism were also providing key substances previously involved in detecting the energetic state of the cell (cAMP, cGMP) and in the synthesis and integrity of membrane systems (IP3, DAG, arachidonic acid, ceramide acid), plus the key enzyme ionic-effector (Ca^{2+}). All of them would be reused inside the eukaryotic signaling pathways as *second messengers* or 'symbolic molecules' to amplify the information flow, conveying integrative messages by diffusion into localized regions of a far bigger cell, in connection with all the new membrane systems, compartments, and inner transportation mechanisms.

Before entering in the analysis of all those different signaling resources, either previously separated or inexistent in the physiology of prokaryotic predecessors, we should discuss about the "why", the evolutionary strategies: Is there a change in the overall functionality signaling systems along that transition why are so important for the development of eukaryotic complexity? What kind of genetic games made possible the evolutionary assembly of eukaryotic signaling systems?

2.1. Recombination as the central evolutionary strategy of signaling systems

The accelerated evolution of the new signaling systems was fundamentally based on processes of *protein-domain recombination*. The amazing signaling novelties of eukaryotic cells, later on excelling in nervous systems, were not due to anyone of the above previous stocks and functional avenues taken in isolation, but to their intercombination in new systems of longer, "mixed" pathways that additionally cross-talked with each other. Osmotic tools (i.e., ion channels) were liberally cobbled together with detection of solutes by protein receptors and with hierarchic chains of protein kinases, as well as with the recycling of proteins in endosomes—finally connecting with ubiquitylation and degradation systems. The postsynaptic processing of the neurotransmitter glutamate appears as one of the best examples of such intercombination of heterogeneous signaling resources (as we will analyze later). The multidomain structure of most of the involved enzymes, proteins, channels, and receptors, in addition to the flexibility of their binding properties made possible the evolution of all those assorted pathways (Pawson and Nash, 2003). In particular, the arrangement of signaling components into integrated *scaffolds*, themselves subject to domain recombination, was a higher-level way to exploit both specificity and convergence of pathways.

Scaffold proteins avoided the entropic penalty for the signaling molecules to find one another in solution; they could also be easily regulated by external signals modifying the association of other proteins with the scaffold, and so they offered a simple, flexible strategy for regulating the selectivity of individual pathways, shaping out signaling regimes and achieving new responses from preexisting components (Good et al., 2011) (see Fig. 1). Being themselves of modular structure, composed of multiple interaction domains and tandem repeat proteins assembled through recombination processes, scaffolds provided an elegant evolutionary strategy for solving the (prokaryotic) signaling dilemma between specificity and co-ordination of the information flows in intracellular networks. In an evolutionary perspective, scaffolds were crucial functional elements to make possible the accelerated process of *meta*-recombination that propelled the assemblage of eukaryotic signaling systems.

However, basic aspects of early eukaryotic evolution are poorly understood yet. Phylogenomic reconstructions show that the characteristic eukaryotic complexity, both in structural and signaling aspects, arose almost "ready made", apparently without any intermediary grades of complexity in between so widely separated levels of organization (Koonin, 2010). Multicellularity and nervous systems were evolved relatively soon, by exploiting, redeploying, and recombining the very communication tools of

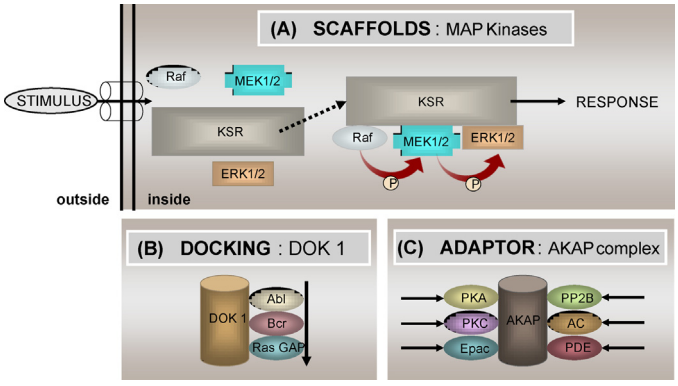

Fig. 1. *Mechanisms of scaffold proteins.* Different classes of scaffolds regulate the dynamics of signaling pathways by binding some of their components and controlling their interaction in complex ways: (a) *Scafolds/anchors* are large multidomain proteins able to bind and regulate multiple components, e.g., MAP kinases cascade. (b) *Docking proteins* have a similar structural and functional outline, but they localize at the membrane next to an activating receptor to which they bind in a phosphorylation-dependent manner, e.g., DOK 1. (c) *Adaptors* are generally small, and have two binding regions to target the action of two bound enzymes and other regulatory proteins. In the entire classes of scaffold proteins, a high level of structural disorder enables key functional attributes such as the binding of multiple enzyme and protein partners.

unicellular eukaryotes (e.g., those already present in yeast). Thus, most components of synaptic pathways in mammals regulating structural plasticity have analogous roles in unicellular responses to environmental cues (ions, nutrients, repellents) and in single cell–cell pheromonal communication (Emes et al., 2008). In point of fact, one of the major evolutionary challenges would be explaining the signaling system of the "last eukaryotic common ancestor" (LECA). How this system was evolutionarily achieved? How many components have been conserved in modern signaling systems?

As new experimental studies and bioinformatic analysis of protein domain architectures have pointed, a number of bacterial and eukaryotic signaling proteins do share similar mechanistic themes (Aravind et al., 2003, 2006; Koonin, 2010). The evidence is that lateral transferred domains of prokaryotic provenance (bacterial and archaea) have also contributed to the evolution of important sensory pathways related to stretch, light, nitric oxide, and redox signaling, plus central developmental paths such as Notch, cytokine, and cytokinin signaling. The phagotrophic life styles of LECA and primitive eukaryotes could have served as conduits for such lateral transfer acquisitions, as well as for the endosymbiotic processes of mitochondria and chloroplasts themselves (Aravind et al., 2003, 2006). In terms of domains and architectures (mechanisms) the parallels between both prokaryotic and eukaryotic signaling systems are startling. However, the intercombination of heterogeneous systems – among the "four evolutionary roots" themselves – and the flexible arrangement of functional modules did allow dramatic expansions of eukaryotic signaling complexity. Besides, the inner mechanisms of control of the new eukaryotic subsystems (nucleus, mitochondria, flagellum, membrane systems, autophagy) provided plenty of molecular resources that could be taken over and incorporated into major classes of signaling pathways; but facultatively, depending on species, tissues, cell types, cell-cycle phases, etc.

Overall, *domain recombination* seems to have been the prevailing genetic "force" or process in the evolution of eukaryotic signaling (respect the four conventional forces acting on genes: mutation, recombination, selection, and drift), even more determinant than in the evolution of prokaryotic signaling systems themselves (Alm et al., 2006). Some additional factors such as the intron–exon organization of genes in eukaryotes, the differential splicing procedures, and the evidence of two episodes of whole genome duplication along vertebrate evolution would have provided far ampler 'computational search' scenarios to exploit domain recombination. Taking into account that more than 45% of the eukaryotic genome is an accumulation of residual transposon and retrotransposon activities, both ancient and recent, this has inevitably conveyed a multitude of possibilities for domain recombination events as well as an increased fine-tuning in their directionality and control (Lander et al., 2001).

Actually, the components of signaling systems (pathways, receptors, signals) have dominated central epochs of multicellular evolution, as recent comparative studies on conserved nonexonic elements show (Lowe et al., 2011). The remarkable expansion of behavioral and cerebral complexity achieved by vertebrates was inseparable of the increasing molecular complexity and sophistication achieved by their signaling systems along these meta-evolutionary processes, comprising also conservation and expansion of a number of *cis* non-coding DNA sequences devoted to fine-tuning of gene control and innovations in posttranslational protein modifiers (Carroll, 2005; Davidson, 2010; Lynch et al., 2011; Lowe et al., 2011). In the case of mammalian brains, recombination strategies seem to have been incorporated even deeper—in ontogenetic development. Recombination via retrotransposons and mobile elements that are active during cerebral development has become a new way to generate additional molecular complexity and *mosaicism*, particularly in the signaling capabilities of cortical and hippocampus' neurons (Lander et al., 2001; Baillie et al., 2002; Kang et al., 2011).

As already said, we will continue with the evolutionary discussion in Section 6, linking it with the informational perspective to elaborate. Let us now examine in more detail the main resources and avenues – the "four evolutionary roots" – that eukaryotic signaling systems have put together, either through direct prokaryotic heredity or through functional co-option along the advancement of modularization.

3. Four evolutionary roots of eukaryotic signaling systems

3.1. The prokaryotic component-systems for "detection of solutes"

The detection of external substances to be processed as signals (rather than as metabolic substrates) occurs in almost all existing prokaryotic cells. Indeed, it may well be considered as the first evolutionary resource in the assemblage of eukaryotic signaling systems. In prokaryotes, a variety of molecular systems are involved in the detection of solutes, ranging from simple transcription-sensory regulators (a single protein comprising two domains), such as the well-known *embR, alkA* or *furB*, to systems of several components and interconnected pathways that regulate key stages of the cell cycle, such as latency, pathogenesis, replication, and dispersion. A basic taxonomy of bacterial signaling systems was proposed by the authors somewhere else (Marijuán et al., 2010), which was centered on "the 1-2-3 scheme" (see Fig. 2):

- The first level of signaling complexity corresponds to simple regulators, "the one-component systems (OCS)." In fact, most cellular proteins involved in cellular adaptation to changing environments, in a general sense, could be included as participants in this primary category (Galperin, 2005). Around one hundred different OCS elements may be present in a moderately complex prokaryotic cell.
- Increasing the scale of complexity, the "two-component systems (TCS)" appear, which include a *histidine kinase* protein receptor and an independent *response regulator*; conventionally they are considered as the central paradigm in prokaryotic signaling systems, and in fact, a number of intercellular communication processes among different species are carried out by these specialized systems. A few dozen TCS pathways may be present in the prokaryote.

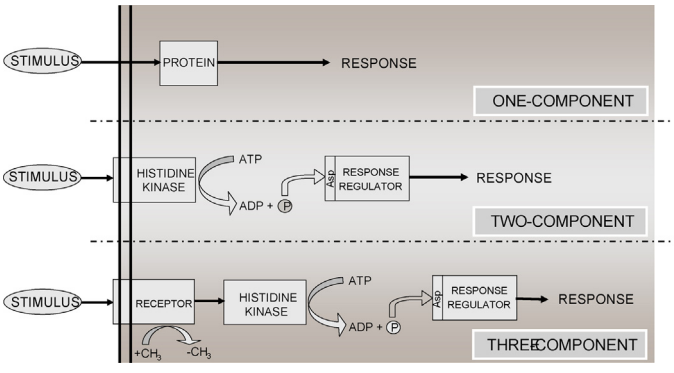

Fig. 2. *The one-two-three component systems (OCS, TCS, ThCS).* These three systems are the characteristic classes of signaling pathways developed by prokaryotes. The external stimulus is perceived either by an internal receptor–transducer (above), or by a transmembrane histidine kinase that connects with a response regulator (center), or by an independent receptor associated to the histidine kinase (below). This scheme represents the basic taxonomy of bacterial signalling; the three different options imply very different information processing capabilities and metabolic costs.

- To maintain conceptual coherence, an additional category, the "three-component system (ThCS)" should apply to two-component systems that incorporate additional non-kinase receptor for activating the protein kinase (e.g., methylated receptors described for the chemotaxis). Very few pathways are showing the ThCS arrangement but they are very important ones (e.g., those devoted to chemotactic guidance) and usually they are subject to further regulations, such as the variable methylation of receptors.

The signaling pros and cons of OCS, TCS, and ThCS (the "1-2-3 scheme") determine their functional deployment. The relative disadvantage of one-component systems stems from the fact that they detect their stimuli almost exclusively in the cytosol (including environmental cues such as light, gases and other small molecules); afterwards they act on DNA-binding more than 80% of cases (Ulrich et al., 2005; Grigoroudis et al., 2007). The evolutionary strategy to overcome the limitations of these single signaling elements has consisted in dividing the individual protein in two halves, putting one of them on the membrane and the other half remaining as a soluble cytosolic regulator, keeping both linked via a phosphotransfer relay (Ulrich et al., 2005). Thereupon, two-component systems (TCSs) appear as a good evolutionary solution to enlarge signaling performances beyond the capabilities (sensitivity, amplification, adaptability) of the single cytosolic receptor–transducer (OCS).

However, it has to be realized that a number of single proteins, acting as OCSs, represent the most abundant signaling strategy in many pathogenic as well as free-living bacteria; that OCS are the most ancient components endowed with such signaling functions; that their signaling dynamics is easily combined among the multiple inner paths; and that they may strongly interact with the other more complex signaling systems—so they are a fundamental factor in the organization of "bacterial intelligence". In point of fact, the relative predominance of the cytosolic – intracellular – mode of detection of OCS versus the extracellular mode of TCS has been used (their ratio) to characterize the "introverts" versus the "extroverts" in the prokaryotes, as well as to gauge the extra metabolic complexities incorporated in the life cycle (Galperin, 2005).

In the transition to eukaryotic signaling, most of the simple OCSs were relegated to strictly metabolic functions; however, cytosolic and nuclear detection by single proteins have been kept for steroid and hormone signaling, among others cases where specificity and security are entirely primed. While not many TCSs remain functionally active as such (mostly in plants and yeasts) they have been massively replaced in animals by "new" serine/threonine and autophosphorilated tyrosine receptors, which are very rare in prokaryotes. The reason is that phosphorylation by histidine protein kinases involves recognition of a three-dimensional folded surface, becoming less amenable to the recombination play than serine/threonine kinases, which recognize linear unstructured motifs as well (Kiel et al., 2010); the same is true for tyrosine kinases' evolvability. Further types of unconventional two component systems exist in bacteria: ECF proteins (related to "sigma factors") and some eukaryotic-like serine/threonine protein kinases and phosphatases mainly used to control cell shape and to interfere with signaling paths of the infected eukaryotic host cells. Interestingly, there seems to be a significant number of three component systems, or ThCS, among the serine/threonine protein kinases of eukaryotic style.

As we will see in the pathways of Table 1, the paradigm of *solute detection* adopted by eukaryotes has contemplated an extraordinary development of enlarged TCSs and ThCSs, involving all kind of assorted pathways. In particular, ligand-binding both to transmembrane receptors and to the *ion channels* counteracting the Donnan effect becomes of outmost importance for the information processing of nervous systems.

3.2. Counteracting the Donnan effect: Molecular mechanisms for "sensing the solvent"

What is called the Donnan effect (or better, the Donnan–Gibbs effect) refers to the osmotic disequilibrium that a living cell experiments by the fact that it contains predominantly negative charges (i.e., nucleic acids' and amino acids' moieties) separated from the liquid environment by a semipermeable membrane. As a result of the opposing osmotic and ionic influences generated, a series of ionic and solvent exchanges follow, without interruption, with the net result that the membrane swells and finally bursts. In point of fact, one of the earliest inventions of living cells was a series of molecular mechanisms to actively counteract the Donnan effect: stretch activated channels, voltage channels, ionic pumps (Na/K), aquaporins, etc. (see Fig. 3). They needed to play with the very factors of the ionic/osmotic disequilibrium so to restore the appropriate levels of membrane mechanical stress, electric potential, and ionic concentrations. Anecdotally, voltage ion channels of bacteria have been finally 'caught in the act' while regulating the membrane potential in recent fluorescence experiments on respiratory metabolism (Kralj et al., 2011).

Neurons turn out to be the greatest specialists in the use of those ancestral solutions, as usual within an ample recombination game, so that they put together an amazing, electromolecular "information processing system" based on the generation and circulation of electric perturbations within a generalized network of membrane potentials. Ion channels associated with (ligand) receptor domains serve as fundamental portals for most neurotransmitters, particularly the fast-acting ones (glutamate, acetylcholine, glycine, GABA). In addition, voltage activated channels of K, Na, Ca, and Cl classes become in charge of integrating and propagating the electric perturbations of the membrane potential across neurites and cell bodies. The kinetic response properties of each channel and its state transitions – as well as its "inactive" transient state – are carefully fine-tuned by means of differential splicing, so that dozens and dozens of slightly different types are deployed as needed in the different neuronal functions and localizations. For instance, there are

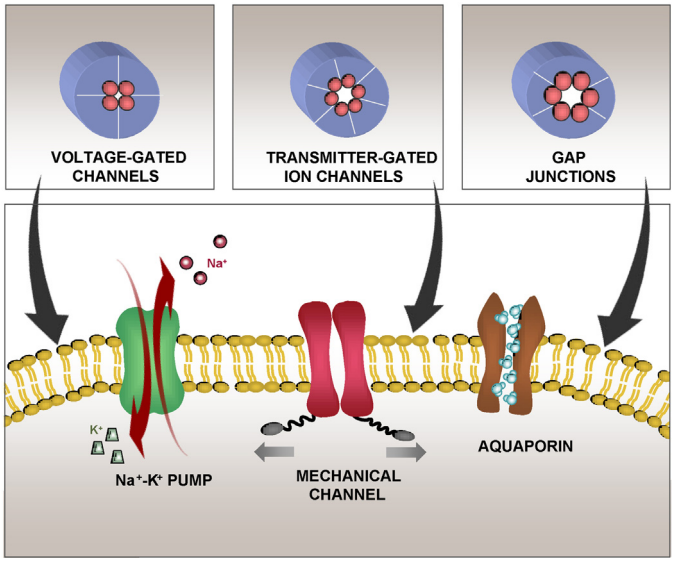

Fig. 3. *Six classes of osmotic related proteins.* A collection of special proteins are responsible for transferring solutes and solvent molecules across cellular membranes. Above, three major classes of membrane channel-proteins: (a) *Voltage-gated ion channels*; (b) *Transmitter-gated ion channels;* (c) *Gap junctions.* Below, another three independent osmotic inventions: (d) *Na-K pumps*, for the unequal exchange of ions and maintenance of membrane voltage; (e) *Mechanical ion channels*, initially for response to mechanical/osmotic forces; (f) *Aquaporins*, for facilitated import and export of water molecules.

over 80 mammalian genes that encode potassium channel subunits, which later on are subject to countless post-transcriptional and functional modifications that create the singular channel exactly needed for each particular tissue or neuronal locus. Overall, permanently keeping the signaling control of electric potentials along the cellular body and the prolongations of neurons is quite strategic as a sophisticate computational resource, but energetically it becomes too expensive. The well-known Na/K pump, part of the prokaryotic stock, is a key element within the osmotic/ionic toolkit upholding the membrane potential of neurons; it represents, however, the highest metabolic expense of the nervous system (up to 2/3 of the total).

Thereafter, cellular and neuronal *excitability* may be deployed as a global physiological property based on the electric field and supporting the fastest responses by animal tissues and organs (appearing e.g., in neuronal, muscular, renal, cardiac and epithelial cells). Basically this property has been built upon the whole electromolecular tools counteracting the Donnan effect. But it is not only cellular excitability that emerges from the osmotic toolkit, a new form of physical detection has also been derived from the very tools used for measuring *osmotic force* (water content), a performance which is fundamental for cell survival. The *mechanosensititive* (MS) ion channels that initially acted as "emergency valves" in primitive prokaryotic cells have evolved in multicellulars, either fungal, plant or animal species, towards an extraordinary range of mechanosensors and transient receptor proteins (TRPs) that detect gravity, growth, blood pressure, muscle stretch, sound, thirst, heat, and so on (Kung, 1990, 2005).

In point of fact, the receptors of sensory neurons in charge of detecting variations of the external (and internal) medium could be broadly classified as based either in *detection of solutes* (vision, smell, taste, hormones, pheromones, nutrients, neurotransmitters, neuropeptides) or in *solvent detection* (sound, touch, pressure, texture, propioception, osmolarity, heat, volume, vibration) (Kung, 1990). In the first case, receptors associated to G proteins are the most frequent detection path, while in the second case mechanosensitive channels and TRPs become the central tools. Mechanical channel-based senses differ from other senses in their close association with the *membrane lipids* (explaining their higher susceptibility to anesthetics) and with the *cytoskeleton*; so they are able to directly sense and transduce force differences into ionic currents derived from the channel opening and closing, and subsequently can also be arranged in a number of detection configurations and signaling paths.

Therefore, in the same way that a number of eukaryotic receptors and pathways could be consistently related with the prokaryotic system for *detection of solutes* (the "1-2-3 scheme"), the eukaryotic variety of sensitive MS channels and TRP subfamilies (TRPV, TRPC, TRPA, TRPP, etc.) could also be consistently related with homologous MS channels of bacteria and archaea for *solvent detection* (Kung, 2005). Those mechanosensitive channels are particularly relevant in epithelial, cardiac, intestinal, and renal tissues, and also in the sensory neurons of advanced nervous systems. The bulk osmotic imprint in eukaryotic signaling pathways is impressive: we have already stated that most of the electrical-signaling mechanisms implementing the information processing of neural cells are directly derived from the osmotic toolkit of prokaryotes.

3.3. The interface between signaling pathways and cell-cycle controlling mechanisms

Discussing the role of the cell cycle in eukaryotic signaling would demand a change of perspective. Unlike the two previous prokaryotic resources, the cell-cycle machinery cannot be considered as a predecessor or as a new, inner prolongation of the signaling system. Rather, the cell cycle appears as the main 'user' of the whole signaling information system, its genuine 'master'—and as such it has finally mixed and hybridized its own organization with the structure of the most strategic signaling pathways, endowing them with the most powerful downstream processing sets. It is as if the cell cycle machinery would have projected itself towards the surface in order to take the most relevant guidance cues from the external environment, subordinating thereafter a number of signaling pathways.

The most powerful set of protein kinases in the whole signaling system is, thus, directly associated with mitotic control: the MAPK cascade (MAPKKK, MAPKK, MAPK). Depending on the cellular context, this cascade will be divided into three branches: MAPK/ERK, SAPK/JNK, p38/MAPK. Whatever receptors and transducers happen to be associated with the successive kinase hierarchies of these cascades, they come to occupy a highly privileged position in the control of the cell cycle and the life and death decisions. And this may happen regarding an ample variety of inputs. Precisely, one of the functions attributed to MAPK cascades is to coherently insert a large variety of cross-talk signaling inputs from other pathways, but endowed with the appropriate relevance and hierarchical order throughout the different signal amplification values of the successive kinase hierarchical levels (see Fig. 4).

A *populational control* of the different phases of the cell cycle (G1, S, G2, M) and their respective transitions takes place along the modular organization of the multicellular organism. Such populational control is ultimately based on a cloud of internal and external signals, usually of opposed signs (activators vs. inhibitors), that carefully regulate the reproductive and specialization trajectories of cells and tissues. These signal coalitions are subtended by the most complex intracellular networks, where inner controlling apparatuses and outer signaling apparatuses turn out to be inextricably mixed with the very complex machinery of the cell cycle. Most of the signaling pathways listed in Table 1 are sending their information to cyclins, MAPK cascades, phosphatases, checkpoints, and other molecular machines and protein complexes involved in the cell-cycle realization, including governance of the apoptotic

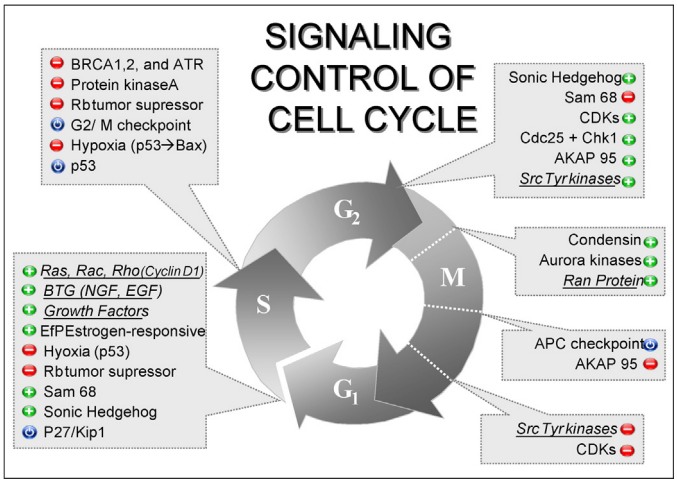

Fig. 4. *Cell-cycle control.* A tight signaling control is established on the different phases of the cell cycle (*G1*, gap; *S*, synthesis; *G2*, interphase gap; *M*, mitosis) and on their respective transitions. The modular organization of the multicellular organism allows the space-time separation between cell-cycle phases, mediated by a number of controlling signaling pathways. The signaling control is ultimately based on a cloud of internal and external signals, usually of opposed signs (activators vs. inhibitors), that carefully regulate the reproductive and specialization trajectories of cells and tissues. In the figure, activating signaling pathways promoting progress of the cell-cycle bear the ⊕ sign, while the inhibiting ones bear the ⊖ sign. In the case of cellular checkpoints the sign is ⊙, as they can result in progress or in arrest, depending on the incoming factors. The signaling pathways associated to MAP kinases appear in italics.

phase. Throughout the final actuators of these pathways, multiple *growth factors* and *apoptotic factors* are secreted locally by neighboring cells, also in a paracrine way, and by the extracellular matrix structures, together enacting the population-based control.

The balance between growth factors and apoptotic factors becomes essential for the developmental and physiological pruning up of the organism. It is this balance what propels cellular growth, eliminates transformed, senescent or redundant cells, and keeps organs and tissues within their functional bounds. And this includes the synapses of the nervous system, behaving as almost autonomous cellular subunits. Thus, the elimination of decaying synapses, as well their maintenance, growth, and motion along the dendritic tree, are strongly related with the balance mechanisms between opposed pathways in the postsynaptic site, which are activated or inhibited along *consonant* or *dissonant* waves of electrical activity in between the synapse and the axon (Section 5.2). As will be discussed later, the multiple signaling pathways and controlling networks implementing those electrical-molecular dialogs at the postsynaptic site involve a formidable complexity, challenging any reasonable description—at least at the time being.

As a sort of reminder of the symbiotic origins of eukaryotes, the control center regarding the irreversibility of death decisions along the cell cycle (once the balance or symmetry between growth and apoptotic signals is broken) locates in mitochondria—the Bcl-2 protein linked with the integrity of the respiratory chain. It makes a lot of evolutionary sense thus, the involvement of respiratory proteins as a form of permanent selection for cells and organisms endowed with the formidable power of mitochondrial symbiosis (Blackstone and Green, 1999; Lane, 2011). The metabolic centrality of mitochondria makes them an important target of a number of signaling pathways, a cross-roads where metabolic state, cell cycle state, and external signals are gauged together, converging in fundamental "checkpoints" where the fate of the whole cell is decided. Notwithstanding their metabolic and cellular importance, mitochondria do not directly provide further molecular mechanisms for downstream signaling components that would branch into other cellular subsystems. The capture and integration of almost all of its genes within the nuclear genome has deprived mitochondria of apportioning their own mechanistic chains for downstream signaling—like in the apoptosis case just mentioned, the involved proteins belong to the host cell.

3.4. Cytoskeleton and endocytic matrix: Signaling incorporation of mechanical and membrane-remodeling systems

A dynamic cytoskeleton and a dynamic membrane are primordial traits of eukaryotes. Probably they coevolved as they functionally need each other—and presumably both derived from the phagocytic life style of LECA and early eukaryotes. With their increasing complexity, these two cellular systems have represented an important source of additional mechanisms for both upstream and downstream regions of numerous signaling pathways.

3.4.1. Endocytosis and the vesicular trafficking of receptors and messengers

Endocytosis is one of the cellular processes more tightly associated with signaling dynamics, "two sides of the same coin" (Scita and Di Fiore, 2010); together they might be conceptualized as a single process that is central to the cellular "master plan" of the eukaryote. The association of signaling molecules (basically receptors, but not only them) with the vesicle dynamics of membranes is the key factor; it is a tightly regulated topological phenomenon that largely constraints the final output of the concerned pathways. In a number of them, recycling of receptors to and from the plasma membrane by means of endocytic and exocytic cycles regulates the presence of receptors and the maintenance of an appropriate level of signaling. AMPA and NMDA glutamate receptors are among the best known instances (Section 5.1). Once established the recycling of receptors, the further arrangement of different endocytic routes is an additional way for determining the net signaling output. For instance, clathrin-mediated endocytosis couples the receptors with recycling and the sustaining of signaling, while non-clathrin mediated endocytosis couples them with degradation (Mills, 2007; Scita and Di Fiore, 2010).

Neurons intensively and regularly have utilized the properties derived from the endocytic matrix. Through membrane recycling, "signaling endosomes" are playing a variety of intraneuronal functions. They replenish the cell surface with ligand-free receptors, resensitize G-protein coupled receptors, provide specific scaffolding for ligands and signaling complexes, generate unique signals prohibited at the plasma membrane, control the trafficking of integrins in cell migration, and associate with rapid microtubule-mediated transport for conveying molecular signals over long distances (Scita and Di Fiore, 2010). Besides, highly complex capabilities of populational communication in the homeostasis between tissues might be mediated by the endocytic matrix through the release of *exosomes*. These are microvesicles emitted extracellularly that in some cases, loaded with hundreds of mRNA and microRNA classes, can reprogram a cell when taken up (Valadi et al., 2007). Maybe, that the cephaloraquidean liquid also serves as a conduit for such extra cellular communication would not be too farfetched a hypothesis.

3.4.2. Cytoskeleton and the signaling incorporation of force fields

Without an active cytoskeleton, endocytosis does not make evolutionary sense, and vice versa. Functionally, they need each other. The eukaryotic cytoskeleton, more than a physical scaffold, corresponds to a dynamic and adaptive structure whose components and regulatory proteins are in a constant flux mediated by a number of intra- and inter-cellular signals (Fletcher and Mullins, 2010). The cytoskeleton spatially organizes the contents of the cell, connects with the forces of the environment, and generates itself coordinated forces that allow movement and change of shape. In much the same way that the expansion of osmotic mechanisms allowed the eukaryotic harnessing of electrical fields and electrical forces, the cytoskeleton represents the cellular harnessing of mechanical forces and force fields for multiple functional purposes. It becomes a high-level integration locus where force fields acting on the cell, biochemical signals, and genetic programs co-determine cell function and fate.

In multicellulars, cell–cell adhesion and contractility (based on the property of *excitability*) emerge as critical determinants of morphological arrangements and motility. Relationships with neighboring cells (cadherins) and with the extracellular matrix (integrins) are covered by dedicated signaling pathways that generate a diverse array of arrangements and morphogenetic events during development, implementing a combinatorial strategy based on relatively few modular components (Montell, 2008) (see Fig. 5). In a middle road between osmotic and mechanical functions, *gap junctions* and *tight junctions* must be cited. The former provide continuity between electrical/ionic/osmotic states of neighboring cells, important for instance in the direct electrical coupling between excitatory and inhibitory neurons; while the latter intervene in the mutual mechanical anchoring between neighboring cells.

In spite of all its apparent complexity, the incorporation of mechanical factors within signaling becomes a simplifying, integrative instance: quasi-instantaneous force propagation nicely complements the time delays of chemical diffusion. Thus, the convergence of functional-signaling modules and cytoskeletal states implies a very strategic asset in order to cohere the cellular relationships with the tissular environment. It is well known how the rigidity and granularity properties of the growth medium are

	PROTRUSION	CONTRACTILITY	CELL-CELL ADHESION	CELL-MATRIX ADHESION
BORDER CELLS (frontiers)	highest	highest	highest	low
CUBOIDAL CELLS (structured)	low	very high	very high	very high
COLUMNAR CELLS (structured)	low	very high	very high	high
SQUAMOUS CELLS (unstructured)	medium	medium	medium	highest

Fig. 5. *Mechanical properties of the cell.* The table represents distinct combinations of mechanical properties that characterize cellular shape and behavior. Varying properties, either qualitatively, or quantitatively, or spatially, will produce a number of arrangements, providing sufficient combinatory complexity to explicate the diversity of existing cellular morphologies—as well as the multiple geometries, topologies, movements, migrations, etc. Different genetic programs may also be activated by the distinct combinations of mechanical properties. (Modified from Montell, 2008).

a direct cause of gene expression and cellular transdifferentiation (Montell, 2008). The mechanical property of *tensegrity* (the dynamic, self-organizing equilibrium based on opposed forces of compression and tension in the cytoskeleton) is another cohesive factor that makes possible the quasi-instantaneous integration of mechanical inputs and outputs as well as the self-regulation of form—and so it has been incorporated into a number of developmental processes and physiological functionalities at the level of cells, tissues, and organs (Ingber, 1998).

It may look surprising that complex neuronal phenomena such as migration, axonal growth cones, axonal inner transportation, synaptic generation, synaptic displacement, and synaptic elimination are tightly regulated by the cytoskeleton-focused signaling paths and their modular combinations. Among the cytoskeletal components of neurons involved, the system of microtubules (MT) has been highlighted as one of the fundamental partners in the learning and memory processes, providing far more than physical support to the ad hoc changes occurring in postsynaptic spines. In developing neurons, actin and MT systems act together to support growth and differentiation of neurites (Section 5.1); and both are of key importance in mature synapses too, supporting the local changes of spines during learning/plasticity and neuronal circuitry remodeling (Jaworski et al., 2000). Bold hypothesis on other computational capabilities of MTs are not new, either in unicellular eukaryotes or in neurons, and some of them invoke the harnessing of quantum phenomena for the sake of cellular computation (Clark, 2010a,b) or for supporting the neuronal emergence of consciousness (Hameroff and Penrose, 1996; Hameroff, 2010).

3.4.3. *The adaptability of signaling configurations*

In the cytoskeleton as well as in the endocytic matrix there is a problem to establish rigorous functional categorizations at the signaling system level. What is the frontier between signaling and the cytoskeleton, or between signaling and endocytosis? The flexible modularization existing in gene networks and protein networks is part of the answer: frontiers depend on the cellular context (Siso-Nadal et al., 2009). We haven't said much about proteolysis, protein degradation, and autophagy either. The autophagy network is one of the biggest and most complex functional systems of the cell (Behrends et al., 2010). Ubiquitylation, the addition of ubiquitin by specialized chains of ubiquitin ligases, has become one of the most abundant protein covalent modifications in signaling proteins, often conducing to degradation in proteasomes; but also to participation in signaling, transportation, and remodeling instances. It is a common way to turn off a temporary pathway once it has accomplished its functional goal—through degradation of an essential component. Quite many other protein modification processes and control subsystems, apart from the omnipresence of phosphorylation, are contributing to signaling configurations: acetylation, methylation, summoilation, glycosilation, lipidation, and so on. While phosphorylation and ubiquitylation occur in the whole cell, acetylation and methylation are common modifications of nuclear proteins (histones), and lipidation and glycolisation are often associated to membrane signaling elements. The protein folding process also provides important mechanistic chains for intracellular signaling. The unfolded protein response (UPR) senses the insufficiency of folding mechanisms, mostly in the lumen of the endoplasmatic reticulum, and puts into action several intracellular signaling branches that activate gene-expression programs to maintain cellular homeostasis or to induce apoptosis if the stress cannot be mitigated (Walter and Ron, 2011).

To emphasize that a number of *intracellular* signaling elements may be incorporated in the canonical and non-canonical *extracellular* signaling pathways herein focused; it is an essential aspect of the signaling system's plasticity, which is mediated by differential splicing, scaffolds, histones' code, gene coactivators, etc. *Molecular recognition* as performed by specific instances (protein domains) turns out to be the fundamental phenomenon supporting all this signaling combinatorics, though it passes unnoticed in most functional analysis (Marijuán, 2001). It depends on the tissues and on the cellular circumstances that a signaling path, or a particular scaffold, will have this configuration or another one, that there will be downstream addition of elements from some other subsystems or not, either totally or just partially. That inherent flexibility, or widespread adaptability, always supported in specific molecular recognition instances and appropriate kinetic regimes, should be a central idea to keep in mind while we list and categorize in next section the most important signaling pathways—the canonical ones. We may say that signaling, like the cell itself, is always in the making, adaptive, anticipatory; changing from tissue to tissue; advancing with the life cycle of the cell and with the development of the tissue; keeping pace finally with the life cycle of the entire organism (Marijuán, 2002).

4. Fundamental signaling pathways in multicellular development and physiology

There are around two dozen major signaling pathways in eukaryotes—the canonical ones. For functional reasons derived from their amazing electro-molecular encoding of memories, it belongs to neurons the signaling privilege of keeping most, if not all, of those pathways in action. In general, while some paths are at work exclusively in the early ontogenetic development, others show up in the organogenesis stage, and others take charge of specialized physiological functions; and there are also specialized pathways surveying the integrity and "healing responses"—inflammation, apoptosis, and necrosis included. It could be speculated that the deployment of all these signaling pathways proceeds by following a signaling "master plan" as we have just discussed for endocytosis (Section 3.4.1). The evolutionary commonality we see at the macroscopic level – structural *bauplans* – could also take place at the level of signaling pathways utilization, and particularly at their functional *co-option*, which necessarily has to be combined with tinkering upon the genetic 'addresses' for transcriptional enhancers (Carroll, 2005; Loehlin and Werren, 2012). Though such signaling master plan is far from being unraveled yet (see the tentative notion of *cellular bauplans* by Mojica et al., 2009), most of the embryonic developmental processes only involve, quite intriguingly, a handful of pathways.

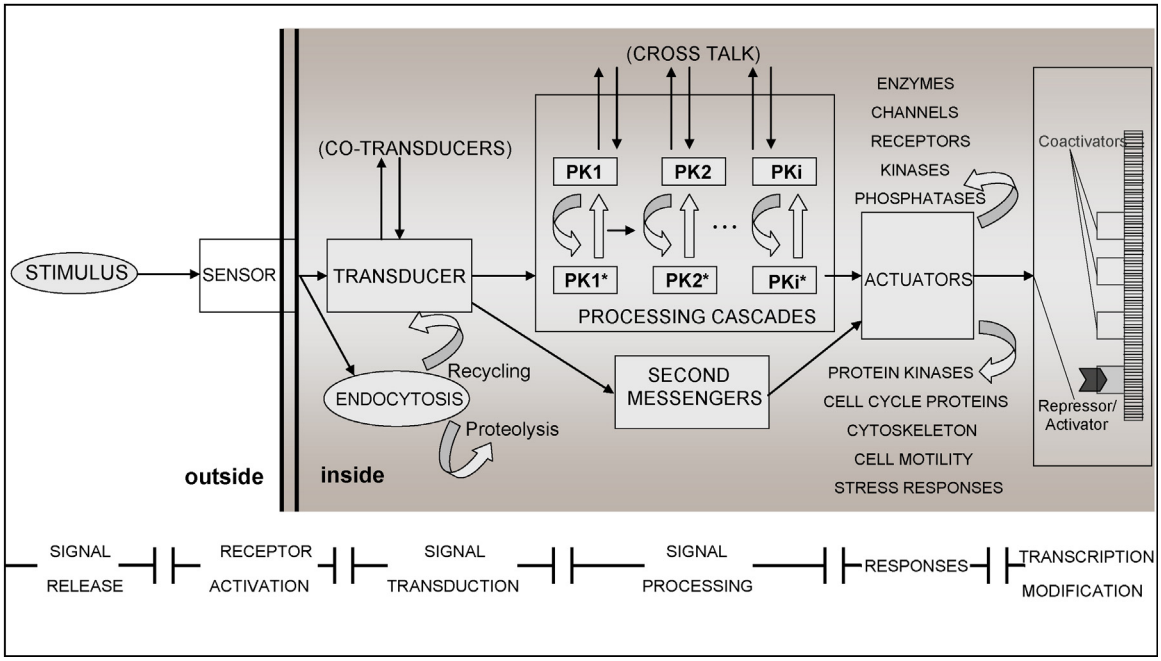

Fig. 6. *Prototypical signaling pathways of multicellularity.* From left to right, a stimulus in the intercellular space binds to a transmembrane receptor (sensor) on its extracellular domain. Upon binding, the receptor undergoes a transient modification of its cytoplasmic domain; this effect triggers a transient modification of a series of proteins in the cell, each one acting as an intermediate in the signal transduction pathway (signal processing), with characteristic hierarchies of protein kinases and second messengers. The last components are actuators or effectors that activate or inhibit proteins and channels that control several cellular functions, notably gene expression by means of transcriptional switches that may interact with several coactivator partners. The whole biochemical changes produced in the cell represent the response to the received signal—its *molecular meaning*.

4.1. The prototypical signaling pathway

What does a prototypical eukaryotic signaling-pathway look like? Fig. 6 captures the most general traits; it might be compared with Fig. 2 about the conventional structure of prokaryotic paths. Functionally, three main features may be distinguished: reception events at the membrane, processing by cytoplasmic mechanisms, and transcription of nuclear targets.

- *Membrane receptors.* External signals, or first messengers, may enter the cell through multiple gates. Via direct membrane crossing, ionic channels (activated by means of voltage, external ligands, internal ligands, stretch), G-protein coupled receptors, tyrosine kinases, serine/threonine kinases, phosphatases, multidomain peptides, and other enzyme-associated receptors. Overall, in the order of two thousand receptors are coded in the human genome, the variety of which is amplified through alternative splicing and post-translational modification. In some cases, an incoming signal may activate different receptors at the same time, crossing to the cytoplasm through different pathways (e.g., steroids, neuromodulators, opiates), each one producing a different effect. In other cases, a branching or "multiplexing" of pathways occurs, and there are several alternative *non-canonical* paths, besides the canonical one, which may be followed by the signal (or better, by its *effects*) after activation of the common receptor; it depends on the cellular context which of these alternative branches, canonical path plus non-canonical ones, will be functionally active or relevant (Scott and Pawson, 2009; Hyduke and Palsson, 2010). Needless to say that the distinction between canonical and non-canonical paths is not always very clear, based often on precedence of discovery.
- *Cytoplasmic transducers and processors.* Like in artificial circuits, variations registered by the receptors have to be filtered, processed, and amplified so that they can convey to downstream effectors an appropriate timing and amplitude of signaling effects. The classical Weber–Fechner law, that external variations of signals are logarithmically transformed by the inner processing mechanisms, seems to be one of the most common sensory outcomes (neurally and otherwise). The very structure of enzyme kinetics – substrate, product, and effector relationships – easily provides logarithmic transformations, but the variety of systems and mechanisms involved is endless: scaffolds, enzyme complexes and networks, phosphorylation cascades, ion-channels, second messengers, endocytosis, proteasomes, cytoskeleton's MTs, microfilaments, etc. Not to mention the retinue of kinases, phosphatases, phospholipases, adenyl- and guanyl-cyclases, phosphodiesterases, cyclins, proteases, caspases, and other enzyme classes eventually involved. Subsequently the dynamic regimes emerging at the end of each signaling pathway, even more taking into account the *cross-talk* between them, do not necessarily keep any formal relationship with the class and intensity of the received signal; the effects of the original perturbation are "systemic" (Liu et al., 2011). In other cases, the recruitment of processing components into scaffolds and protein complexes may prevent cross signaling between pathways or may commit highly multifunctional molecules to very particular functions (Good et al., 2011).
- *Nuclear targets.* One of the essential consequences of signaling is the specific activation of gene targets by downstream effectors working as transcription factors. At this "final" transcriptional stage is where most of the commonalities among pathways occur. There are specific signaling pathway response elements (SPREs) which perform as *transcriptional switches*, whereby target genes become activated in the presence of signaling elements but repressed in its absence. *Default repression*, *activator insufficiency*, and *cooperative activation* constitute the central principles of transcriptional-signaling control, shared among the major pathways (Barolo and Posakony, 2002; Pires-daSilva and Sommer, 2003). In general, the SPRE-binding transcription factors are converted from default repressors to activators upon arrival of the

signal. Default repression refers to the transcriptional repression of target genes in the absence of signaling. Fig. 6 shows how the arrival of the final response element of the pathway transforms the standing transcription factor and activates it. The presence of numerous coactivators working in complex "logical machines", enacting combinatorial regulation of transcription, ensures that the expression activity is restricted to specific territories or groups of cells, or functional contexts (Davidson, 2006, 2010). In addition, there has to be a previous arrangement of the euchromatin–heterochromatin expression state, also regulated via signaling pathways impinging on the *histone code*, so that the cooperative activation of the transcriptional switch will lead to appropriate gene expression. Epigenetic mechanisms are integrated here, acting as permanent markers on DNA itself (C methylation), accompanied by different, more transient modifications in the amino acids of histones, such as *Lys* acetylation, *Lys* and *Arg* methylation, *Ser* phosphorylation, and *Lys* and *Arg* ubiquitylation. This capability to selectively prepare the expression state of chromatin regions becomes one of the essential aspects of the signaling system's guidance.

4.2. The catalog of eukaryotic signaling pathways

In what follows (see Table 1) we have worked upon Gerhart's scheme (1999), modifying it to include new categories such as stress and criticality, electromolecular classes of osmotic mechanisms, and some new pathways (Hippo, complement cascade, apoptosis). The exuberant diversity of paths, the multiple variations and assortments in special cell types, the facultative mixing with multitude of inner control mechanisms, the mechanistic complexity of most of the pathways themselves, conspire all to preclude the development of all-encompassing and meaningful classifications.

Table 1 lists the major pathways (21 of them) and highlights a few mechanisms and functionalities. It contains a highly abbreviated description with emphasis on aspects related to nervous system's functioning. The following main functional categories have been distinguished:

- *Early development*: Wnt, Hedgehog, Notch, TGF-β, Neurotrophins.
- *Mid development and organogenesis*: Integrins, Cadherins, Nuclear Hormones, Reelin.
- *Tissue physiology*: G-Protein Coupled Receptors, Guanylate Cyclase, Electromolecular Transmission (4 main classes).
- *Stress and criticality:* NFkB, Cytokines, Autophagy, Apoptosis, Hippo, Complement Cascade.

None of those pathways acts in isolation. Cells simultaneously receive many extracellular stimuli, and through cross-talk between the diverse signaling paths activated, they may process and interpret multiple inputs *differently* even in slightly different contexts. For instance, a developing neuron exposed to two agonists, each fostering a mutually exclusive response such as growth or apoptosis, must decide which signal to follow. This decision will occur, for instance, by means of a third substance tipping the balance between the two pathways (Section 3.3), with one of the pathways interacting with the other and shutting down its activity thereafter.

A series of balances and symmetry breakings between opposed pathways are systematically crossed along the processes of development, morphology, and physiology. Frequent players are Wnt and Hedgehog, Hippo and Wnt, Notch and Hedgehog, Hippo and TGF-β, etc. Such balances and asymmetrical interconnections between pathways, far from being linearly organized, are enmeshed in networks and circuits of fiendish complexity. Cell polarity, referring to the appearance of two distinct poles as a result of asymmetry along a particular axis, becomes a regular building block to generate asymmetry in further tissues and organs (Li and Bowerman, 2010). One of the most important developmental steps in the embryo refers to what is called the epithelial–mesenchymal transition (EMT), which is critical for the formation of many tissues and organs as well as for physiological processes such as wound healing and initiation of metastasis in cancer. As a result of this transformation, well-stacked epithelial cells lose their polarity and adhesion-junctions and gain invasive and migratory capabilities becoming mesenchymal cells. Processes such as gastrulation, neural tube formation, heart formation, as well as different types of cancer occur in dependence of those signaling pathways, basically a series of inputs and outputs around the Par complex signaling, involving Wnt, Notch, TGF-β, Tyrosine kinases, and the cytoskeleton (McCaffrey and Macara, 2011). In other cases, mesenchymal cells experiment the reverse process (MET) in order to participate in the formation of many epithelial mesodermal organs. The flexible conjugation of both EMT and MET events is an essential feature of the developmental process.

In multicellular – vertebrate – development, signaling is everywhere: from the earliest steps of axis specification, to the diverse kinds of morphogenesis, organogenesis, and growth in the embryo; from sexual maturation and regular tissue renovation to the ongoing physiology in the adult (Gerhart, 1999). Actually, each phylum is characterized by a body plan, *bauplan*, which is a unique topological configuration of secreted signals, active signaling pathways, and expressed genes, all of them dynamically self-organized along the development and life cycle of the individual. We have already mentioned that a signaling master plan could be envisioned; but it could hardly take any formal expression. Nevertheless, some tools for symmetry and asymmetry compositions in group theory could provide some help in the theoretical-biological approach to developmental symmetry-breaking processes (Leyton, 2001). The cancer genome landscapes dramatically show how mutations in multiple components of the developmental signaling pathways (in development and tissue differentiation: Ras, MAPK, TGF, Notch, Wnt, etc.) create havoc in the tissular and organismic order.

Perhaps the best way to characterize signaling complexity is to go to those tissues where most signaling pathways may be caught into functional action. Neurons, for instance, which contain a hypertrophy of signaling elements above any other eukaryotic tissue or cellular specialization; they are the best specialists in the use of extra/intracellular computing. Table 1 makes clear that almost ALL signaling pathways are potentially involved in one or another aspect of nervous system development, structure or function. Having to deal with the most subtle biological stuff, *information*, and having to organize a macroscopic system for memory storage, has probably represented the highest evolutionary challenge and has involved the most complex, sophisticate signaling solutions. The molecular machinery subtending memory, mostly in the postsynaptic site of excitatory neurons, is perhaps one of the best instances of such evolutionary signaling complexity. It will be briefly described in the following section.

5. Signaling complexity in synapses—The molecular seat of memory

The concept of the synapse is closely associated with the 'advanced' nervous systems of vertebrates. However, "protosynapses" are ancestral eukaryotic traits – close to LECA origins – that have evolved further structural and functional complexity as their aggregate information processing characteristics increased in sophistication along the modularization process of multicellulars (Ryan and Grant, 2009). From temporary portals where information relative to metabolic and reproductive states of unicellular partners was exchanged, synapses have evolved towards stable adhesive junctions between cells across which information is relayed by directed secretions irrespective that electromolecular

phenomena may participate in the exchanges or not. Actually the cells of both the nervous system and the immune system have relied on synapses as a central communicative tool. In each of these systems, synaptic types are built around a microdomain structure including central active zones of endocytosis and exocytosis surrounded by adhesion domains. Both systems have produced an ample variety of synaptic types, based on specific molecular recognition events, intercellular adhesion mechanisms, positional stability and directed secretion for communication (Dustin and Colman, 2002; Steinman, 2004). Among them, the highest degree of functional organization and structural complexity corresponds to neuronal excitatory synapses.

5.1. Molecular structures in the postsynaptic site—Spines

In the dendrites and soma of pyramidal neurons, most of the synapses are excitatory and release the neurotransmitter *glutamate*; dendritic *spines* are the corresponding postsynaptic contact sites. Characteristically, the spine morphology consists of an expanded head connected to the dendrite shaft by a narrow neck. Within their tiny dimensions, about 1 to 3 μm long and 1 μm diameter, spines encircle an astonishing complexity: the postsynaptic proteome reaches more than 1000 different protein components in mammals, while the presynaptic proteome comprises several hundred proteins (Emes et al., 2008; Ryan and Grant, 2009).

On the presynaptic side, a secretory apparatus is assembled which is activated by appropriate electromolecular signaling events (incoming action potentials). Correspondingly, on the postsynaptic side a receptor surface is put in place containing molecular machinery that transduces the neurotransmitter secretory signals liberated in the synaptic gap into activation of relevant intracellular signaling pathways. There is evidence that cadherins and neuroligins, among other partners, are functioning as synaptic specifiers at the molecular recognition phase of synaptogenesis, as well participating as adhesive struts in gluing together pre- and post-synaptic sides across the cleft (Cohen-Cory, 2002).

Most of the postsynaptic processing machinery is contained as a highly organized ensemble attached to the membrane, the *postsynaptic density* (PSD). This superstructure encloses a number of scaffolds and protein complexes associated to different glutamate receptors and to actin microfilaments; it also incorporates relevant enzymes, proteins, and ion channels belonging to several major pathways (Baron et al., 2006; Okabe, 2007). Stable microtubules do not directly enter into spines, but dynamic ones with their EB3 associated proteins seem to be participating in the organization of synaptic plasticity, as they are required for controlling the levels of F-actin within spines and are thus essential for the maintenance of spine morphology and maturation (Jaworski et al., 2000).

It is in the signaling machinery at the postsynaptic density where most of the synaptic plasticity needed for information processing and the formation of memories is established—usually, under the form of long term potentiation and long term depression, LTP and LTD, respectively (Newpher and Ehlers, 2009; Murakoshi and Yasuda, 2012). The most intensively studied instances of LTP and LTD dynamics correspond to the hippocampus CA3-CA1 glutamate excitatory synapses. Three main classes of glutamate receptors have been identified there: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or "quisqualate") plus the associate KA (or "kainate"), NMDA (*N*-Methyl-*D*-aspartate), and mGluRs (metabotropic glutamate receptors) (see Fig. 7).

The highly complex subcellular distribution of glutamate receptors, tightly controlled, is central to achieve a synaptic regulation in dependence of the activity produced. Many relevant molecular agents participate in PSD regulatory complexes, some of them are PI3K, Rac, Rap, non-receptor tyrosine kinases, etc. Of outmost importance are *neurotrophins*, a family of neuronal

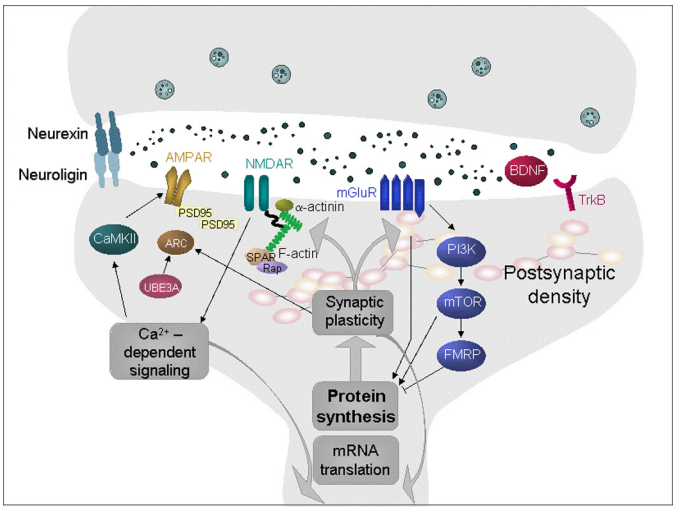

Fig. 7. *The postsynaptic density.* Most of the postsynaptic processing machinery is contained in a highly organized ensemble attached to the membrane, the *"postsynaptic density"* (PSD). This superstructure encloses a number of scaffolds and protein complexes associated to the different glutamate receptors and to actin microfilaments; it also incorporates relevant enzymes, proteins, and ion channels belonging to several major pathways. Signaling from stimulated glutamate receptors (AMPAR, NMDAR, mGluR) regulates mRNA translation, protein synthesis, and essentially the long-lasting forms of synaptic plasticity. The calcium flux, inducing calcium-dependent signaling networks, triggers further signaling events to the nucleus, leading to the modification of transcripcional regulators and resulting in the induction of activity-dependent gene expression. Genes induced by neuronal activity (such as BDNF, ARC and Ube3A) function to control synapse formation, maturation, elimination, and plasticity.

growth factors that include NGF, BDNF, NT-3, and NT-4/5, among others, which are recognized by receptor tyrosine kinases (see Table 1 and Annex). Neurotrophins were initially characterized as growth factors promoting neuronal survival and differentiation, but they also participate in important aspects of synapse development and function, including the regulation of activation-induced plasticity. Besides, they deeply influence axonal and dendritic morphology through their effect in cytoskeleton components as well as in other signaling pathways. The main interrelationships involve three different pathways that converge on MAP kinases: the AMPA-NMDA system, adhesive neurexins/neuroligins, and actin/MT cytoskeletal components. Protocadherins mediating dendritic self-avoidance as well as many other signaling pathways related to neuromodulators, pheromones, interleukins, and death factors, also participate in spine regulation (Manabe, 2002; Blitzer et al., 2005; Silver and Kanichay, 2008; Newpher and Ehlers, 2009; Murakoshi and Yasuda, 2012; Lefebvre et al., 2012).

If all this signaling complexity was not sufficient it has been accompanied by an unexpected phenomenon: local protein synthesis within the spine itself.

5.2. Local protein synthesis in spines—Molecular markers of plasticity

Among the many puzzles in synaptic plasticity, one of the most relevant is how the activation and calcium influx through NMDARs can give rise to opposite results (LTP vs. LTD) and even conduce to synapse elimination. Just changing the relative timing of pre- and postsynaptic activation by a few tens of milliseconds, the direction of synaptic modification is reversed. Synaptic remodeling becomes one of most complex electro-molecular phenomena. Apart from all the signaling pathways already discussed and some other regulators of receptor complexes (i.e., calcineurin and specialized phosphatases), it demands the local involvement of ubiquitin and proteasomes, and the apportion of a variety of molecular

components (Bingol and Schuman, 2006; Haas et al., 2007; Paolicelli et al., 2011; Hughes, 2012). Numerous switching mechanisms seem to co-operate at different levels: signaling, structural, degradative, transcriptional, and *translational*. The latter implies not only central protein synthesis: the presence of local protein synthesis in postsynaptic spines has also been authenticated.

A variety of tagging molecules are acting as relatively permanents markers to indicate synaptic fates to the mostly blind crews of self-construction and repair mechanisms. Several candidates for tagging have already been proposed: second messengers, protein kinases, adhesion molecules, polymerized actin, and dynamic MTs. Essentially the synaptic tagging oriented to translation would organize the release of mRNAs from specific granules in dendritic accumulation sites and its capture by highly stimulated synapses (spilling the effects to local neighbors). Thereafter in situ protein synthesis would take place. It is by means of this local protein synthesis that some of the molecules needed for increased synaptic transmission are afforded to the proper places. Different patterns of activation would lead to the release of different kinds of mRNAs and to their correlated specific protein synthesis (plus the accompanying proteasome function), either consolidating or weakening the existing synaptic structures (Blitzer et al., 2005). Given that some of the synthesized proteins would contribute to further protein synthesis, a positive feedback mechanism is set up maintaining the newly set synaptic strength within an elaborate space-time framework for the appropriate distribution of the computational/memory effects (Bingol and Schuman, 2006; Silva et al., 2009; Ho et al., 2011; Murakoshi and Yasuda, 2012). Fig. 7 captures this basic scheme.

As a matter of fact, synaptic spines possess the fundamental equipment required for protein synthesis, from ribosomes and mRNA transport to articulate membranous systems, as well as numerous molecular components belonging to the protein translational machinery. All of these have been localized in spines. Synaptic protein synthesis is quite plausible thus; and becomes a parsimonious hypothesis that helps to bring coherence to disparaging explanations on the vastly different mechanisms involved in the computational synapse. It also makes sense in the light of behavioral and ecological evidences on how different time scales and rhythms should be involved in learning consolidation, in the efficient allocation of synaptic memory resources, and in the organization of behavioral cycles (activity, fatigue, rest, sleep). The results from these relatively recent studies are likely to have a considerable impact on neurocomputing itself and on the neurological understanding of memory disorders (Destexhe and Marder, 2004; Feldman and Brecht, 2005; Blitzer et al., 2005; Silva et al., 2009; Ho et al., 2011).

The occurrence of protein synthesis in postsynaptic structures was quite an unexpected finding. Symbolically, one of the most basic and 'primitive' characteristic of life, protein synthesis, makes itself present in one of the most sophisticate evolutionary achievements, the synapse, where it plays an essential role in support of informational process—under the functional guidance of a motley crew of signaling pathways.

6. Final discussion: Signaling and the abstract organization of cellular problem solving

In all biological systems, from prokaryotes to eukaryotes – and rather astoundingly even within neuronal synapses themselves – signaling is tightly coupled with gene transcription and protein synthesis. Theoretically, is there any fundamental link between signaling systems and the basic eukaryotic organization/evolution towards increased complexity?

An immediate rationale is that the transcriptional machinery, being 'blind', needs massive signaling guidance in order to deploy the adequate genetic circuits, so to fabricate and put into the cellular milieu the adequate RNA and protein agents. Thus, signaling means the *topological governance* of the transcriptional regulatory network, the decision of what parts should be activated or should be inhibited, particularly throughout the very fast changes in second messenger concentrations. Herein there is an important quantitative difference between prokaryotes and eukaryotes. In our previous study of prokaryotic signaling (Marijuán et al., 2010) we had argued about the approximately *quadratic* relationship existing between total number of genes and signaling components in prokaryotic species (Ulrich et al., 2005). In the extent to which eukaryotic genomes contain roughly 10 times the number of genes of prokaryotes, an exponential increase in the number of eukaryotic signaling components should not be an unexpected result. A similar increase occurs in the number of genes expressed by the final effectors/transcription factors of each signaling pathway: from around 300 and 400 genes expressed by the cAMP binding element in prokaryotes (*Mycobacterium tuberculosis* and *Escherichia coli*, respectively) we jump to around 4000 genes expressed by the similar CREB protein in humans.

Symbolically, the "1-2-3 scheme" of signaling component systems in prokaryotes (as described in Marijuán et al., 2010) have been replaced by the eukaryotic 21-some major pathway classes. Say, from just three pathways classes to around two dozen ones. This augmented signaling capability is an essential trait of eukaryotic complexity, both structural and functional. It conflates with another important factor: the improved nuclear organization and differential splicing machinery. From an informational point of view, the cell's self-constructing machinery may be seen as a realization of von Neumann's theory of self-constructing machines (Vedral, 2010), which mandates separation between the inner description of the system and its production structure. In the evolution of eukaryotes, that separation is instantiated by means of ad hoc compartments and transportation mechanisms. They make possible longer eukaryotic proteins (50% on average than prokaryotes), most of them multi-domain, which are synthesized following more complex production processes that include splicing, folding, transportation, degradation, etc. Around those very processes a new evolutionary strategy has been enacted—a biomolecular 'engine' of quasi-universal problem-solving capabilities.

The new cellular engine is built to exploit the whole different molecular stages in the protein production processes (transcription, folding, transportation, modification, complexes, degradation), which often are specifically coded onto DNA addresses of different domains. They may be used themselves as new functional elements of control—including the cognate DNA sequences they recognize. Thereafter, cellular solutions may be obtained from very different biomolecular components at different production stages, forming an augmented set of solutions that are separately tinkered with on different protein domains and become linked together onto the same molecule, but facultatively, in a variety of ways involving either alternative splicing, or RNA interferences, or new scaffold arrangements (Maniatis and Tasic, 2002; Good et al., 2011). The evolutionary problem solving game of domain recombination at the DNA level, facilitated by the exon/intron arrangement, has been incorporated, thus, at new functional levels beyond random variations in the reproduction process itself, acting now during development and differentiation, as well as during physiological regular functioning. The signaling system, being in charge of controlling both transcriptome and proteome, masterminds the whole distribution of the problem-solving strategy for each cell type.

A nontrivial aspect of the new eukaryotic encoding strategy is that it parallels the inner processing scheme of today's computers—which is also a von Neumann's legacy. The codes of primary functions as well as the codes of secondary functions (production stages) of multi-domain enzymes and proteins are

put together onto the same DNA memory bank, to be played with phylogenetically and ontogenetically, as well as developmentally and physiologically. In a similar way, the codes of logical functions and memory addresses are also placed together, side by side, into the CPU memory of computers to be played with by the programming instructions (Navarro et al., 2010). While natural computing systems rely on massive parallelism, the artificial ones rely on the highest velocities for sequential processes. In addition, we have already argued that every possible molecular recognition event directly amenable to DNA encoding or indirectly controllable by DNA sequences has been susceptible of incorporation into the computational scheme of eukaryotic cells: noncoding DNA, introns, lncRNA, miRNA, circRNA, distant CIS sequences, topological domains, mobile elements, transposons, retrotransposons, and epigenetic heredity—most of them deployed under direct control of signaling system pathways (Fedoroff, 2012).

It is by means of this whole informational organization—not very well understood yet–that the eukaryotic system has acquired a new degree of "cellular intelligence" and that quasi universal problem-solving capabilities have emerged for the development of complex multicellular organisms. Whereas the limited problem-solving capabilities of prokaryotes have been mostly addressed towards the direct solution of *molecular assimilation* problems (in their encounter with environmental substances), in multicellular eukaryotes there is a conquest of developmental complexity, a quasi-universal solution of *molecular organization* problems. The component cells of multicellular organisms may enact choreographies perfectly organized: moving, changing their shape, sensing chemicals, reacting to forces, playing with electrical fields, feeling specific contacts, reproducing, migrating, differentiating, committing apoptosis... under the continuous guidance of the signaling system. The evolutionary complexification of neuronal signaling in the postsynaptic proteome, together with the local incorporation of protein synthesis, is perhaps the most impressive instance (Ryan and Grant, 2009).

Biological evolution means two basic characteristics: self-production and communication with the environment. Both aspects are irremediably linked within the basic cell-engine of eukaryotic complexity, and the knowledge on both has increased dramatically during last decades. It is in this sense that an informational updating of the venerable "cellular theory" seems possible and necessary. Although interpreting cellular organization in computer terms is far from new, see pioneering discussions in Waddington (1968–1972), and a number of analogies with Turing machines and operating systems have been made recently (Wolfram, 2002; Danchin, 2003, 2009; Yan et al., 2010; Tang and Lyons, 2012), the present schemes are far from satisfying. A new informational approach to the self-production and communication processes of living cells, to the informational organization of both prokaryotic and eukaryotic 'intelligences' looks feasible. Many different strands have to be put together, from open systems, to self-organization, to informational architectures of molecular encoding, self-production, problem-solving engines, signaling guidance... but it looks a plausible task not far from several multidisciplinary enterprises of yesteryear: artificial life, natural computing, synthetic life, or bioinformation. Hopefully, some of the above ideas might contribute to advance the discussion and would provide useful insights.

Acknowledgments

The authors very much appreciate the careful inspection made by one of the anonymous reviewers. His criticisms and suggestions have greatly contributed to improve the quality of the present paper. It is a remarkable review effort that also contributes to maintain the high standards of this veteran journal.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2013.06.005.

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